

Comparison of average estimated metabolic rates for styrene in previously exposed and unexposed groups with pharmacokinetic modelling

Yue Wang, Lawrence L Kupper, Agneta Löf, Stephen M Rappaport

Abstract

Objective—To understand whether previous styrene exposure increases the human liver's ability to convert styrene into styrene oxide.

Methods—The hypothesis was tested that the average linear metabolic rate constant κ was the same in both exposed and unexposed groups, when the exposed group comprised people with a history of styrene exposure and the unexposed group had no exposure. In an experimental chamber, these two groups of subjects were exposed to a concentration of 80 ppm styrene for two hours. A three compartment pharmacokinetic model was used to define κ . Based on large sample theory, the comparison of estimated mean values of κ in the exposed and unexposed groups was shown to be equivalent to a comparison of the estimated mean values of the hepatic clearance X in the two groups. A method was developed to estimate X for each subject in both groups from the subject's height, weight, and estimated asymptotic styrene decay constant α . Here, α was estimated individually from observed blood concentrations over time when sufficient time had elapsed after the controlled exposure.

Results—The proposed methodology of comparing the estimated mean values of κ in exposed and unexposed groups reduced the number of specific physiological variables involved to three, all of which were estimable from data based on simple direct measurements. In contrast, other methods based on pharmacokinetic models usually involved many variables that were non-estimable on an individual basis. Consequently, statistical comparisons were impossible. These methods were applied to analyse previously published data on the time course of styrene concentrations in arterial blood of subjects in both exposed and unexposed groups. A Wilcoxon non-parametric rank sum test with the individually estimated X values was used, and no significant difference in the means of X in the two groups was found.

Conclusion—The linear metabolic rate constant κ for humans is probably not altered by previous exposure to styrene. This result is in agreement with some experimental studies on animals. However, in the data analysis, it was noted that the number of subjects in each

group was small (6-7) and that the styrene concentration data did not exactly reflect true behaviour of asymptotic decay. Further studies are still needed to draw more definitive conclusions.

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Most genotoxic effects of styrene are likely to be due to styrene-7,8-oxide (SO),¹ which is a labile intermediate metabolite of styrene activated by microsomal enzymes.² This is subsequently detoxified into styrene glycol,² which is then oxidised to mandelic acid and phenylglyoxylic acid,³ both of which are excreted in urine. There is speculation that previous exposure to styrene may induce CP450 enzymes and thereby increase the biotransformation of styrene into SO. Andersen *et al*⁴ found that the blood styrene concentrations were lower in rats that had been previously exposed to styrene. From this they reasonably concluded that styrene induced its own metabolism. Elovaara *et al*⁵ showed that styrene metabolism in vitro by liver microsomes was greatly increased after a period of exposure to styrene (2100 mg/m³ for 24 hours) in rats. On the other hand, Mendrala *et al*⁶ found that monooxygenase activity was not changed in liver microsomes of rats and mice that had been exposed for six hours a day for three days to 600 ppm styrene. Likewise, Filser *et al*⁷ exposed rats and mice to 150 and 500 ppm styrene on five consecutive days (6 h/day) and found no change in the rate of styrene metabolism compared with non-pretreated controls.

Unlike in animal studies, direct measurements of metabolic activity are difficult to make in humans. This paper develops the theoretical framework for testing the null hypothesis that the average level of metabolic activity is the same in groups with and without previous exposure to styrene. Then the methods are evaluated with data from Wigaeus *et al* and Löf *et al*,^{8,9} who exposed groups of people with and without previous exposure to styrene at 80 ppm for two hours in an exposure chamber. Arterial concentrations of styrene were measured both during and after exposure. To measure the level of metabolic activity, we present a three compartment pharmacokinetic model to simulate the uptake, distribution, metabolism, and elimination of styrene and to define the linear metabolic rate constant κ . We show that the estimated

Department of
Biostatistics

Y Wang
L L Kupper

Department of
Environmental
Sciences and
Engineering, School of
Public Health,
University of North
Carolina, Chapel Hill,
NC 27599, USA

Y Wang
S M Rappaport

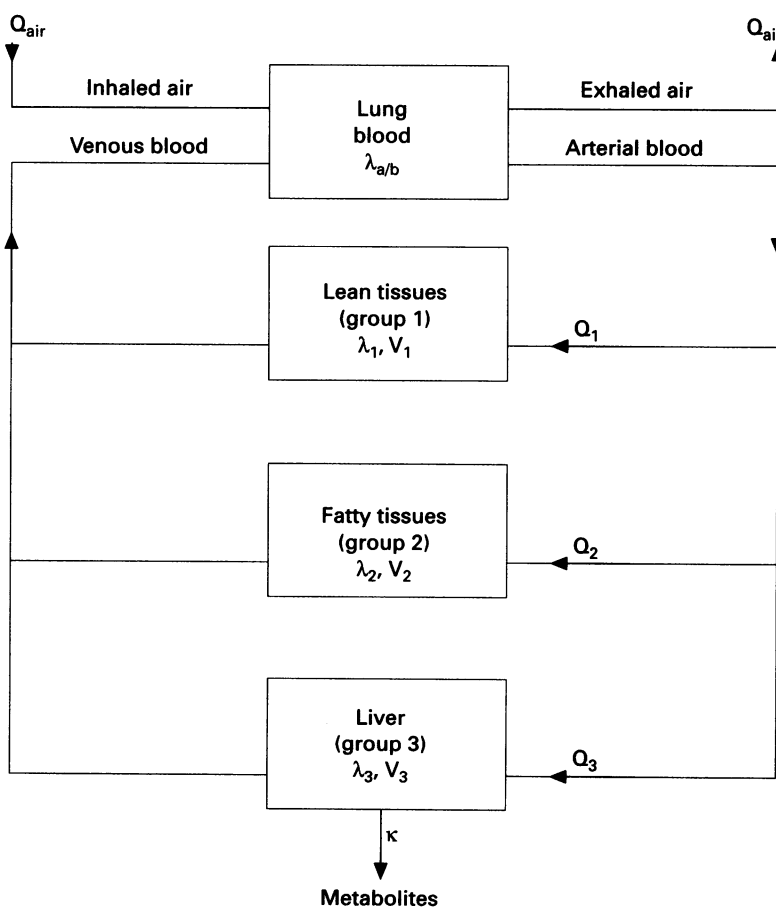
National Institute for
Working Life, S-171 84
Solna, Sweden
A Löf

Correspondence to:
Dr S M Rappaport,
Department of
Environmental Sciences and
Engineering, School of
Public Health, University of
North Carolina, Chapel Hill,
NC 27599, USA.

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mean value of κ in each group is proportional to the estimated mean value of the hepatic clearance X (defined in equation (6)) in that group. When the number of subjects in each group is large, the proportionality constants for the two groups become close to one another. Consequently, for large samples, we can test our null hypothesis through a comparison of the estimated mean values of X .

Normally, to estimate κ and other variables, the use of a physiologically based pharmacokinetic model requires detailed information, such as tissue blood flow rate, cardiac output, tissue volume, ventilation, styrene concentration in blood over time, etc. Unfortunately, most of these input variables are not directly measurable and are replaced by some standard values that are not specific to each person (see table 3). As a result, variables like κ are generally not uniquely estimated, and hence the precision of such estimates is unknown. Our proposed methodology avoids many of these drawbacks. We derive a simple estimator for X from the asymptotic behaviour of the styrene concentration in blood after exposure. This estimator involves only three estimable physiological variables specific to each person, namely, height, weight, and the asymptotic decay constant α , where α is estimated from the decay of styrene concentrations in blood after the end of exposure.



Physiologically based three compartment model. Under moderate physical exercise, the body tissues can be grouped into well perfused tissues (lean tissues), poorly perfused tissues (fatty tissues), and the liver (the main metabolising organ). The time dependent behaviour of styrene concentration in blood is determined by a set of differential equations based on mass conservation. These equations involve the physiological variables shown here.

Methods

DESCRIPTION OF THE MODEL

We assume that the metabolism of styrene takes place exclusively in the liver, although some studies suggest that pulmonary tissues and bone marrow may also participate to a small degree.¹⁰ Under moderate physical exercise, the skin and muscle tissues become well perfused, as do blood vessel rich tissues like the brain, kidney, and viscera. Therefore, the body tissues can be roughly grouped into lean tissues ($i = 1$), fatty tissues ($i = 2$), and the liver ($i = 3$) (figure). We assume that each type of tissue has similar composition—that is, the percentages of lipid and water—and similar blood perfusion, and can, therefore, be characterised by a single tissue/blood partition coefficient λ_i and a single blood flow rate Q_i (in l/min). Throughout this paper, unless otherwise specified, time is in minutes, volume in litres, and concentration in mg/l.

For $i = 1$ and 2, we have a simple mass balance equation:

$$\frac{dC_i(t)}{dt} = \frac{Q_i}{V_i} \left[C_{art}(t) - \frac{C_i(t)}{\lambda_i} \right] \quad (1)$$

where $C_i(t)$ and $C_{art}(t)$ are, respectively, the styrene concentration in the i -th tissue group and in the arterial blood, and where V_i is the effective volume of the i -th tissue group. For the liver, we have an additional term to represent the metabolism of styrene:

$$\frac{dC_3(t)}{dt} = \frac{Q_i}{V_i} \left[C_{art}(t) - \frac{C_3(t)}{\lambda_3} \right] - \kappa C_3(t) \quad (2)$$

where the metabolic rate has been assumed to be first order, as characterised by the constant κ (min^{-1}).

ASYMPTOTIC VARIABLES

During the period after exposure, the blood concentration is found to decline in two phases: a first distribution phase, in which concentrations of styrene in all tissues except the fat come into rough equilibrium with the arterial blood concentration, and a second phase, in which the primary mechanism of the decay is metabolism. Once equilibrium throughout the body tissues has been more or less established, the whole body can be treated as a single compartment.¹¹ Then the equation of conservation of mass gives:

$$\sum_{i=1}^3 \frac{dC_{art}(t)}{dt} V_i \lambda_i = -\lambda_{a/b} C_{art}(t) Q_{air} - \kappa V_3 \lambda_3 C_{art}(t) \quad (3)$$

where Q_{air} denotes the alveolar ventilation and $\lambda_{a/b}$ the air/blood partition coefficient.

We solve this equation for $C_{art}(t)$ to obtain:

$$C_{art}(t) = C_0 \exp(-\alpha t) \quad (4)$$

where C_0 is a constant independent of t , and where:

$$\alpha = \frac{\lambda_{a/b} Q_{air} + \kappa V_3 \lambda_3}{\sum_{i=1}^3 V_i \lambda_i} \quad (5)$$

is the decay constant for styrene concentration in blood. We can estimate α by fitting

Table 1 Styrene concentration ($\mu\text{mol/l}$) in arterial blood at various times (min) after 2-hour exposure to 80 ppm styrene for groups with and without earlier exposure to styrene (heights and weights of all subjects are presented)

Time	Group without earlier exposure to styrene								Group with earlier exposure to styrene							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
75	1.45	1.29	—	2.97	3.91	2.85	2.86	1.94	1.17	2.2	1.91	2.0	3.92	3.33	1.44	1.54
90	1.1	0.82	—	3.28	2.15	2.65	2.16	1.36	1.14	1.91	2.34	2.13	2.6	1.92	1.39	1.38
105	1.16	1.01	—	—	1.69	2.02	—	2.09	1.38	—	1.23	1.27	2.38	1.92	0.71	1.33
120	0.45	0.53	—	2.44	1.35	1.45	1.4	1.76	0.77	1.78	1.25	1.09	1.56	1.29	0.9	1.35
180	0.35	0.51	—	1.42	1.52	1.49	1	2.41	—	—	—	—	—	—	—	—
240	0.54	0.38	—	1.2	1.04	0.82	0.98	1.07	—	—	—	—	—	—	—	—
Height (m)	180	178	180	174	178	191	172	179	182	174	188	173	176	170	180	179
Weight (kg)	80	68	70	72	60	79	68	67	69	72	105	74	72	69	68	78

equation (4) to the final portion of the decay curve with least squares method. Note that the styrene concentration in arterial blood should be equal to that in the venous blood after equilibrium is reached. With this estimated $\hat{\alpha}$ we can then estimate the hepatic clearance (l/min):

$$X = \kappa V_3 \lambda_3 \quad (6)$$

as

$$\hat{X} = \hat{\alpha} \sum_{i=1}^3 \hat{V}_i \lambda_i - \lambda_{a/b} \hat{Q}_{air} \quad (7)$$

(note that the hepatic clearance defined in (6) is not the "apparent clearance" defined in Wigaeus *et al*⁸). As the partition coefficient λ_2 (adipose tissue/ blood) is about 50 times as large as λ_1 (lean tissue/blood) and is over 4000 times as large as $\lambda_{a/b}$ (see table 3)³, the dominant contribution in equation (7) comes from the adipose tissues. Hence:

$$\hat{X} \approx \hat{\alpha} \hat{V}_2 \lambda_2 \quad (8)$$

STATISTICAL COMPARISON

Suppose we have a random sample of subjects of size n_j from the j -th group, where $j = 1$ pertains to the group with previous exposure to styrene and $j = 2$ to the previously unexposed group. All physiological variables vary from person to person. In a sense, each such variable can be considered to have a distribution determined by its varying values across a population of subjects. For subject k ($k = 1, \dots, n_j$; $j = 1, 2$), we can estimate the hepatic clearance $X_k(j)$ according to equation (8). The unbiased estimators of the mean and variance of the underlying distribution of hepatic clearance values for the j -th population are:

$$\hat{E}(X(j)) = \frac{1}{n_j} \sum_{k=1}^{n_j} \hat{X}_k(j) \quad (9)$$

$$\hat{V}(X(j)) = \frac{1}{(n_j - 1)} \sum_{k=1}^{n_j} [\hat{X}_k(j) - \hat{E}(X(j))]^2 \quad (10)$$

where $j = 1, 2$.

As the linear metabolic rate constant κ measures the proportion of the amount of styrene in a unit volume of liver that can be converted to SO in a unit of time, it is therefore independent of the liver's volume V_3 . From equation (6), we obtain:

$$\hat{E}(\kappa(j)) = \frac{\hat{E}(X(j))}{\lambda_2 E(V_3(j))} \quad (11)$$

where $j = 1, 2$, and where it is assumed that κ and V_3 are independent for each subject. If n_j ($j = 1, 2$) is large, we expect that both $E(V_3(1))$ and $E(V_3(2))$ would approach the common population mean $E(V_3)$. Consequently, a comparison of the means of the metabolic rate constants in the two groups can be made through a comparison of the corresponding means of the estimated hepatic clearances. When sample sizes are small, the above statement is still approximately correct if one can ignore any difference in the means of V_3 in the two groups.

STUDIES OF PEOPLE WITH AND WITHOUT PREVIOUS EXPOSURE

Wigaeus *et al*⁸ and Löf *et al*⁹ carried out a study to compare, among other things, the concentration of styrene in arterial blood between groups with and without previous styrene exposure. The unexposed group was composed of eight healthy male volunteers without a history of styrene exposure, whereas the exposed group was composed of eight workers routinely exposed to styrene. All subjects in both groups were exposed to styrene at a concentration of 80 ppm for two hours during light physical exercise at a work load of 50 W. The concentration of styrene in arterial blood was measured several times for each subject during and after styrene exposure. Each subject's height and weight were also measured. Table 1 shows the raw data that are used in this investigation.

Results

Table 2 shows the estimated adipose tissue volume \hat{V}_2 , decay constant $\hat{\alpha}$ and clearance \hat{X} for each subject in the two groups. \hat{V}_2 is esti-

Table 2 Estimates of the decay constant $\hat{\alpha}$ (min^{-1}), adipose tissue volume \hat{V}_2 (l), and the hepatic clearance \hat{X} (l/min) of all subjects in groups with and without earlier exposure to styrene

Subject	Group with earlier exposure			Group without earlier exposure		
	\hat{V}_2	$\hat{\alpha}$	\hat{X}	\hat{V}_2	$\hat{\alpha}$	\hat{X}
1	8.4	0.0053	3.1	24.2	0.0072	12.3
2	17.5	0.0046	5.6	9.7	0.0068	4.6
3	52.0*	0.0116	42.8	11.0	—	—
4	20.8	0.0132	19.4	17.5	0.0067	8.2
5	16.2	0.0193	22.2	—0.9*	0.0025	—
6	16.1	0.0208	23.7	15.7	0.0051	5.6
7	8.4	0.0142	8.4	13.5	0.0033	3.1
8	22.2	0.003	4.7	7.7	0.0027	1.4
Mean	15.7†	0.0115†	12.7†	14.7‡	0.0053‡	5.5‡

*The estimate may not be valid because the subject's height or weight are outside the range over which the regression model (12) is constructed.

†Subject 3 is excluded.

‡Subjects 3 and 5 are excluded.

Table 3 Data used in the standard pharmacokinetic model

	Rest	Workload 50W
Total cardiac output (l/min): Q	6.0 ¹⁹	9.9 ²⁰
Local blood flow to tissue group 1 (l/min): Q ₁	4.1 ¹⁹	8.0*
Local blood flow to tissue group 2 (l/min): Q ₂	0.32 ¹⁹	0.32*
Local blood flow to tissue group 3 (l/min): Q ₃	1.5	1.5*
Volume of tissue group 1 (l): V ₁ ^{3,19}	37.3	37.3
Volume of tissue group 2 (l): V ₂ ¹⁹	14.5	14.5
Volume of tissue group 3 (l): V ₃ ³	1.7	1.7
Tissue/blood partition coefficient for group 1: λ ₁ ³	1.2	1.2
Tissue/blood partition coefficient for group 2: λ ₂ ³	71.0	71.0
Tissue/blood partition coefficient for group 3: λ ₃ ³	2.6	2.6
Air/blood partition coefficient: λ _{air} ³	1/59	1/59
Alveolar ventilation (l/min): Q _{air}	4.9 ¹⁵	20.0 ²⁰

*Assume that the excess cardiac output relative to the rest state will only go to the muscle group.

mated based on a person's height H (m) and weight W (kg) according to the formula:

$$\dot{V}_2 = 1.32(W - 26.69H^{4.24}) \quad (12)$$

This estimator is based on a modification of Döbeln's work,¹² where the product of the sums of right and left femoral condylar breadth and bistyloid radioulnar breadth were replaced by a constant; the factor 1.32 is used to convert the amount of fat (kg) to the corresponding volume (l). The decay constant α for each subject is estimated by fitting equation (4) to the observed blood concentration decay curve from $t = 75$ to 120 minutes (three to four data points) for the treatment group and from 90 to 240 minutes (four to five data points) for the control group. The clearance is estimated with equation (8), with the partition coefficient λ_2 taken from table 3.

As we are not willing to make any assumptions about underlying population distributions and as group specific sample sizes are small, we used a Wilcoxon non-parametric rank sum test to test for a significant difference in the means of the estimated clearance values in the two groups. We obtained a P value of 0.17, implying that the difference in the estimated means of X in the two groups is not significant. Note that the P value for a two sample t test is 0.12 (variances are not significantly different between the two groups: $P = 0.09$), which is in agreement with the non-parametric Wilcoxon test. In both tests, we excluded subject 3 (adipose tissue volume was not estimable from equation (12)) in the group with previous exposure, and subjects 3 (experimental data was not available to allow estimation of α) and 5 (adipose tissue volume was not estimable from equation (12)) in the group without previous exposure. Therefore, the total number of subjects were seven and six in the groups with and without previous exposure to styrene, respectively.

Discussion

Filser *et al*⁷ showed that linear metabolism was operative in rats and mice exposed to styrene up to 1.28 mg/l (300 ppm). Mendrala *et al*⁶ estimated the maximum rate of metabolism V_{\max} (mg/min) and the Michaelis-Menten constant K_m (mg/l) in humans, *in vitro*. In that case, the estimate of K_m was 9.4 mg/l. Löf and Johanson¹³ showed that metabolic saturation did not occur at concentrations below 250

ppm styrene (1.06 mg/l) in human volunteers. Therefore, equation (2) is appropriate for the subjects in our comparison who were exposed to 80 ppm of styrene. However, if the exposure concentration was higher than about 250 ppm of styrene, the equation would be modified to account for saturable metabolism; that is:

$$\frac{dC_3(t)}{dt} = \tau_3 \left[C_{arr}(t) - \frac{C_3(t)}{\lambda_3} \right] - \frac{V_{\max} C_3(t)}{V_3(K_m + C_3(t))} \quad (13)$$

In this case, the linear asymptotic behaviour (3) where

$$\kappa \cong \frac{V_{\max}}{V_3 K_m}$$

no longer holds and equations (4)–(6) would not apply.

It is also possible to estimate κ from normal pharmacokinetic models¹⁴ and then make a comparison of the estimated mean of κ among subjects in the two groups to assess the appropriateness of our proposed methodology. As the partial pressure of styrene in the lung equilibrates within a fraction of a second with that in the alveolar air, the quantity of styrene entering the pulmonary compartment by inhalation and venous blood equals that leaving the compartment by exhalation and arterial blood. Therefore, we have:

$$C_{arr}(t) = \frac{Q_{air} C_{inh}(t) + \sum_{i=1}^3 Q_i \frac{C_i(t)}{\lambda_i}}{Q + Q_{air} \lambda_{air/b}} \quad (14)$$

where $C_{inh}(t)$ is the concentration of styrene in the inhaled air and Q is total cardiac output. The effective tissue/blood partition coefficient for each tissue group is the volume weighted average of each individual tissue partition coefficient in that group:

$$\lambda_i = \frac{\sum_j \lambda_{ij} V_j}{\sum_j V_j} \quad (15)$$

For instance, the lean tissue group in our model can be divided into two tissue subgroups: the blood vessel rich tissues and the skin and muscle. The volumes of blood vessel rich tissues and the skin and muscle tissues have been estimated³ to be 7.1 and 36.3 litres, respectively, while the tissue/blood partition coefficients for styrene have been estimated to be 2.54³ and 1.0,¹⁵ respectively. Therefore, we obtain $\hat{\lambda}_i = 1.2$ from equation (15). As most physiological variables cannot be directly measured on an individual basis, some population standard values are usually used. Table 3 lists all those literature based standard values that are needed in this model. Now, κ can be estimated by fitting the model (equation (1), (2), and (14)) to the experimental data on styrene concentrations in arterial blood. We find many possible estimated values for κ . The range of our possible estimates of κ could be as large as 20-fold when allowing those standard values to vary over reasonable ranges.¹⁶ This undesirable phenomenon has been recognised by

many others—for example, Watanabe *et al.*¹⁷ As with any compartmental model, the uncertainties associated with these standard values will necessarily lead to uncertainties about the key variables. Therefore, valid statistical comparisons cannot be made based on these estimated values of κ .

The error involved in estimating α from our data might be large because the observations were obtained in only two to four hours after the end of exposure. Ramsey *et al.*¹⁸ monitored the decay behaviour of styrene in blood of four male volunteers over 47 hours after exposure to 82 ppm styrene for six hours. The half life for the terminal decay phase was measured to be 13.0 (± 0.7) hours, which translates into an α value of 0.053 (± 0.003) h⁻¹. These values are about 1/12 and 1/6 of those we estimated for the groups with and without previous exposure to styrene, respectively. Therefore, the decay constants of Wigaeus *et al.*⁸ and Löf *et al.*⁹ may indeed be too large to represent the real terminal decay behaviour.

Strictly speaking, equation (8) cannot be used if α is derived from the decay behaviour well before equilibrium has been established throughout the body, including the fat tissues. This condition is rarely met rigorously in practice and may constitute the limitation of the application of this method. However, in the event that this equilibrium was not achieved in this study, we think that our conclusion that styrene exposure did not increase metabolic activity would still be valid as the shorter period of observation for the subjects with previous exposure relative to those without (2 h v 4 h) would have exaggerated the differences in κ values between the two groups (equation (11)).

This result disagrees with that of Löf *et al.*⁹ We think that the main reason for this discrepancy is the higher volume of distribution of styrene in the pre-exposed subjects due to their larger amounts of adipose tissue. As we stated, the decay constants estimated by Wigaeus *et al.*⁸ and Löf *et al.*⁹ were likely to be more overestimated for the pre-exposed group than for the group without previous exposure. As a result, the estimated areas under the blood concentration v time curves for the two groups would tend to be significantly different. However, as the data set is small and as the data on styrene concentration do not exactly reflect the true asymptotic decay behaviour, we think that further studies are needed to draw more definitive conclusions.

Finally, we will make a few comments about our estimation of κ . Taking $\alpha = 0.053$ h⁻¹ by Ramsey *et al.*¹⁸ and other data from table 3, we find from equation (8) and (11) that $\kappa = 0.2$ /min. Mendrala *et al.*⁶ measured the Michaelis-Menten constants V_{\max} and K_m associated with styrene with human livers in vitro to be 3.2 mg/h/kg body weight and

0.09 mmol/l, respectively. From equation (13), this translates into a κ value of about 0.2/min when assuming 70 kg body weight and a liver volume of 1.7 l (see table 3) for a standard man. Therefore, our estimate agrees very well with the experimental results obtained from human livers in vitro. In contrast, κ would be 2.57/min by scaling up the variables for rats,¹⁵ and κ could vary anywhere from 0.1/min to 3.0/min with least squares methods when allowing 30% variation for the standard physiological variables. This again points to the desirability of estimating human kinetic variables from human data rather than from extrapolation based on animal experimental data.

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